Immunoglobulin E-Binding Structures on Antigen-Presenting Cells Present in Skin and Blood

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In atopic individuals, cutaneous antigen-presenting cells (APC), i.e., Langerhans cells and dermal dendritic cells, frequently display anti-IgE reactivity. Although earlier observations suggested that this phenomenon results from the binding of (complexed) IgE to the low-affinity IgE receptor (FcεRII/CD23), we and others demonstrated recently that Langerhans cells, dermal dendritic cells, and peripheral blood monocytes from atopic individuals can bind monomeric IgE via the high-affinity receptor for IgE (FcεRI). These new observations re-stimulated investigations aiming to unravel the nature and functionality of the relevant *in vivo* IgE-binding moiety(-ies) on APC. New data demonstrate that FcεRI, both quantitatively and qualitatively, is the pivotal serum IgE-binding structure on APC of atopics and, even more important, that FcεRI on APC functions as an allergen-focusing molecule. Thus, it is likely that allergens may be more efficiently taken up, processed, and presented to T cells after targeting to APC via FcεRI as compared with allergen binding to APC in the conventional manner. *In vivo*, FcεRI-IgE–dependent allergen presentation may critically lower atopic individuals' threshold to mount allergen-specific T-cell responses. This would result in the perpetuation of allergen-specific IgE production (type I reactions) and perhaps even the occurrence of T-cell–mediated, delayed-type hypersensitivity reactions in allergen-exposed tissues. *J Invest Dermatol 104:707–710, 1995*

The syndrome of atopy consists of three major symptoms, i.e., allergic rhinoconjunctivitis, allergic asthma, and atopic dermatitis [1], and is usually associated with elevated serum immunoglobulin E (IgE). Whereas the pathogenetic role of allergen-specific IgE is clearly established in the case of allergic rhinoconjunctivitis and allergic asthma, the manifestation of atopic dermatitis cannot be easily explained by the occurrence of type I allergic immune reactions. In fact, the clinical and histopathologic picture as well as the emergence kinetics of atopic eczema roughly follow the criteria of delayed-type (type IV) immune reactions. It is presently unclear whether IgE-mediated (type I) and T-cell–mediated (type IV) allergic reactions of atopy are events occurring independently of each other or are pathogenetically linked.

IgE-BINDING STRUCTURES ON LANGERHANS CELLS AND DERMAL DENDRITIC CELLS

The first evidence for a possibly causative role of IgE in the pathogenesis of atopic dermatitis was derived from studies showing that skin from patients with atopic dermatitis harbors IgE^+ dendritic cells in the epidermis (Langerhans cells) as well as in the dermis (dermal dendritic cells) [2,3]. Initial attempts to characterize the critical IgE-binding structure on Langerhans cells suggested that these cells express the low-affinity IgE receptor (FcεRII/CD23) *in situ* [4] and that this receptor is up-regulated after exposure of Langerhans cells to certain cytokines [5]. However, the failure of specific anti-CD23 monoclonal antibodies (MoAbs) to block entirely IgE binding to Langerhans cells [4], the obvious high affinity of this IgE-binding structure, and the fact that Langerhans cells of both normal and atopic skin are capable of IgE binding [2,6] prompted the search for IgE-binding moieties other than CD23. Recently, this issue was clarified by the demonstration that epidermal Langerhans cells and dermal dendritic cells of healthy persons bind monomeric IgE via the high-affinity IgE receptor FcεRI [6–9], previously thought to be expressed exclusively on mast cells and basophils. Recently, our group has shown that the high-affinity IgE receptor FcεRI is the biologically relevant IgE-binding structure on epidermal Langerhans cells and dermal dendritic cells in diseased atopic skin (Klubal R *et al*, in preparation). The functional significance of FcεRI expression on dendritic antigen-presenting cells (APC) is only poorly understood but could be similar to that observed for other FcRs on APC, i.e., the binding of monomeric or antigen-complexed Ig followed by signal transduction and internalization of FcR-bound immune complexes [10–12]. More rapid progress in this research has been hampered by the relative sparsity of these cells and by difficulties in isolating and purifying them from tissue samples. This problem may soon be circumvented by experimental protocols allowing the *in vitro* generation of Langerhans-cell–like dendritic cells from the pool of CD34-expressing stem cells from cord [13] and peripheral blood (Strunk D *et al*, submitted) or by the cytokine-driven expansion of FcεRI-expressing dendritic cells from as yet poorly characterized CD34-negative precursor cells present in human peripheral blood [14,15]. In the meantime, a valuable experimental tool for the investigation of FcεRI function on APC was gained by our recent finding that Langerhans cells are not the only APC capable of FcεRI expression.

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IgE-BINDING STRUCTURES ON MONOCYTES/MACROPHAGES

Until recently, it was generally believed that monocytes/macrophages express only low-affinity IgE binding sites, i.e., the inducible form of the low-affinity IgE receptor CD23 [16] and the IgE-binding protein ϵBP [17]. This led to the assumption that these cell types preferentially bind preformed IgE complexes rather than monomeric IgE. We have found recently that monocytes of atopic individuals can bind monomeric IgE and that this binding occurs via FcϵRI [18]. In particular, monocyte FcϵRI expression was detected in most patients with atopic dermatitis (70% to 80%) and in a proportion of those with allergic rhinoconjunctivitis (approximately 50%). However, this demonstration of FcϵRI on monocyte surfaces does not exclude the possibility that, under *in vivo* conditions, the binding of polyclonal serum IgE to monocytes occurs predominantly via FcϵRII or ϵBP. We have addressed this issue experimentally and have found that freshly isolated monocytes of atopics, but not of nonatopics, carry cell-surface–bound IgE *in vivo* and that the majority of IgE-binding sites are occupied by (elevated) serum IgE (Maurer D *et al*, submitted). The further observation that lactic acid treatment but not lactose treatment of the cells almost completely removed *in vivo*-bound IgE molecules argued against a possible role of ϵBP in this process, but did not exclude an important role for CD23 as a relevant IgE-binding molecule *in vivo*. For this purpose, we exposed lactic-acid–treated monocytes to sera from birch-pollen–sensitized individuals, either in the presence or absence of inhibiting MoAbs to FcϵRI or CD23, and visualized the binding of birch-pollen–specific serum IgE by a subsequent incubation step with biotinylated recombinant *Bet v* I, the major birch-pollen allergen. The MoAb to FcϵRI selectively abolished the binding of polyclonal serum IgE to monocyte surfaces (Maurer D *et al*, submitted). These results indicate that, in analogy to Langerhans cells, monocytes of allergic individuals carry FcϵRI-bound, allergen-specific IgE *in vivo* and that the interaction with polyvalent allergen is followed by the triggering of FcϵRI rather than CD23.

STRUCTURAL AND FUNCTIONAL ANALYSIS OF FcϵRI ON APC

On basophils and mast cells, FcϵRI is expressed as a tetrameric holoreceptor composed of one α-chain, one β-chain, and one γ-chain homodimer [10]. Molecular and biochemical studies have revealed that Langerhans cells [6,7] and monocytes of atopic persons [18] co-express the IgE-binding FcϵRIα-chain and the signal-transducing FcϵRIγ-chain at both the mRNA and protein levels and that the monocyte FcϵRIα and FcϵRIγ protein chains are physically associated and thereby form functional FcϵRI complexes (Fiebiger E, unpublished observations). For reasons yet unknown, our attempts to detect FcϵRIβ mRNA and protein in Langerhans cells and monocytes have so far been unsuccessful [6,18]. Apart from procedural considerations, it is possible that the high-affinity IgE receptor on APC consists of α- and γ-chains only. In fact, transfection experiments have shown that this chain composition suffices for functional cell-surface expression of the human receptor [19,20]. However, the COOH-terminal intracytoplasmic portion of the β-chain has been found to contain a conserved protein sequence motif (antigen recognition activation motif) [21], which allows the physical association with members of the src protein tyrosine kinase (PTK) family. Accordingly, recent findings have re-emphasized an eminent functional importance of the β-subunit, showing that the constitutively β-associated src PTK lyn is crucial for phosphorylation and activation of the FcϵRIγ subunit. FcϵRIγ thus modified can then activate the PTK syk and initiate further downstream signaling events, i.e., phosphorylation and activation of phospholipase $C_{\gamma 1}$, breakdown of phosphoinositols, and elevation of the cytosolic calcium concentration [22]. Despite the apparent lack of β, FcϵRI on APC is capable of mediating PTK activation [23] and calcium mobilization [18,23], indicating competent signaling via this receptor. At the moment, it is tempting to speculate about the presence of a putative β-like structure that functionally substitutes for the "classic" FcϵRIβ-chain in APC.

BIOLOGIC CONSEQUENCES OF ALLERGEN-IgE BINDING TO FcϵRI ON APC

The binding of allergen-specific IgE to FcϵRI on mast cells and basophils, followed by bridging of the membrane-bound IgE molecules by soluble, multivalent allergens, results in cellular degranulation and consequently in the release of proinflammatory substances such as histamine, serotonin, prostaglandins, and leukotrienes [24–26], as well as in the synthesis and secretion of cytokines such as interleukin (IL)-3, IL-4, IL-5, IL-6, granulocyte–macrophage colony-stimulating factor, and interferon-γ [27–29]. Because FcϵRI on APC constitutes a functional cell-surface receptor and APC are potent producers of cytokines [30–34] and eicosanoids [35,36], it is likely that FcϵRI induces the release of biologic effector molecules by APC and critically modulates or even initiates allergic inflammatory responses in allergen-exposed tissues.

We recently found that, besides its putative cytokine-inducing properties, FcϵRI on professional APC functions as an allergen-focusing receptor structure. Using sera from grass- or birch-pollen–sensitized donors and recombinant birch (r*Bet v* I) and grass pollen (r*Phl p* II) allergens as well as hapten (hydroxy-nitrophenacetyl, NP)-specific, monomeric IgE (cIgE) and NP-conjugated allergens, we observed that the presence of cell-surface–bound IgE results in efficient allergen binding to the monocytes of atopics. MoAb-blocking experiments revealed that FcϵRI, rather than FcϵRII, is the pivotal moiety that mediates this event. Even more important, cIgE-mediated allergen binding to monocyte-enriched, peripheral blood mononuclear cells of atopic persons resulted in a 100–1000-fold amplification of r*Bet v* I or r*Phl p* II presentation to autologous T-cell clones with peptide specificities for *Bet v* I or *Phl p* II. The addition of the anti–FcϵRIα-chain MoAb, but not of an anti-CD23 MoAb, reduced this cIgE-enhanced, allergen-specific T-cell–clone response to levels seen in the absence of cIgE. This demonstrates that FcϵRI, but not FcϵRII, mediates IgE-dependent allergen uptake, processing, and presentation by professional APC from atopics (Maurer D *et al*, submitted). Thus, our findings emphasize an as yet unknown function of FcϵRI in atopic diseases and provide clear evidence that the presence of this receptor on APC critically lowers the threshold of atopic individuals to mount allergen-specific T-cell responses.

With regard to IgE-mediated cutaneous allergy, these findings may have pathogenetic significance for delayed-type atopic inflammation occurring in the skin and, perhaps, in other tissues. If repeated allergenic exposure of the skin and/or mucosal tissue results in the production of allergen-specific IgE **(Fig 1*A*)**, then the interaction of allergen with IgE bound to FcϵRI on skin APC may result in IgE-facilitated allergen presentation to T cells in lymphoid and non-lymphoid organs and, furthermore, in allergen/IgE-dependent activation and cytokine secretion by FcϵRI-expressing cells within the tissue **(Fig 1*B*)**. In particular, monocytic cells secrete IL-1 and tumor necrosis factor-α after cross-linking of cell-surface–bound IgE moieties *in vitro* [34]. These two cytokines have been shown to play an indispensable role in the elicitation of cutaneous late-phase atopic reactions [37,38]. IL-1 and tumor necrosis factor-α exert some of their biologic effects via induction of E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 expression by endothelial cells [37,39]. Up-regulated endothelial cell adhesion molecules promote and reinforce leukocyte–endothelial-cell interactions and, therefore, are a prerequisite for transmigration of inflammatory cells into inflamed tissue [40]. In delayed-type atopic reactions, the latter event is characterized by extravasation of (Fc-IgE receptor-bearing) eosinophils and macrophages, as well as by a pronounced accumulation of T lymphocytes [3]. At this particular step, IgE-amplified allergen presentation by FcϵRI-expressing skin APC may decisively control the quality and quantity of allergic tissue inflammation. Even in the presence of minute allergen concentrations, this mechanism may allow effective activation and clonal expansion of skin-infiltrating,

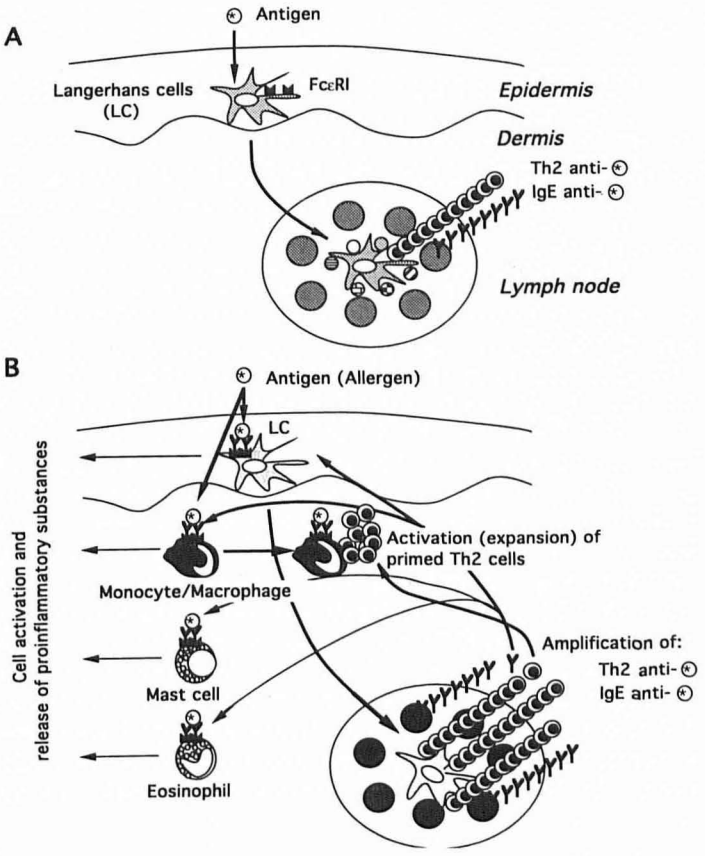

Figure 1. Hypothetical model of pathogenetic mechanisms operative in cutaneous delayed-type atopic inflammation. *A*, repeated allergen exposure and genetic factors are implicated in the preferential expansion of allergen-specific ThO cells and in their conversion into Th2-like cells. As a consequence, allergen-specific B cells differentiate into plasma cells that produce allergen-specific IgE. *B*, Upon allergen re-exposure, IgE-bound skin APC (Langerhans cells and other dermal dendritic cells) may be directly activated by allergen-mediated cross-linking of their high-affinity IgE receptors (FcεRI). This may be followed by the induction of cytokine production and secretion and, as a consequence, may result in increased expression of adhesion molecules on dermal endothelial cells and in the influx of eosinophils and T cells. On the other hand, even in the presence of minute allergen concentrations, FcεRI-mediated amplification of allergen presentation may result in the efficient activation of allergen-specific Th2 cells, which cause allergic tissue inflammation due to cytokine release.

allergen-specific T helper cells, with a Th2-like cytokine secretion pattern [41–43] and with the capacity of mediating (IL-4–dependent) allergic tissue reactions [44]. Moreover, activated allergen-specific Th2-like cells should possess the capability to promote B cells to secrete allergen-specific IgE, which again binds to FcεRI-expressing APC in skin and other tissues. If this allergen-driven, self-amplifying mechanism is operative in atopic diseases *in vivo*, then therapeutic strategies should aim to interrupt this vicious circle by interference with FcεRI expression, IgE binding to FcεRI, and/or FcεRI-mediated signal transduction by APC.

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REFERENCES

1. Coca RF, Cooke RA: On the classification of the phenomena of hypersensitivities. *J Immunol* 8:163–182, 1923
2. Bruynzeel-Koomen C, van Wichen DF, Toonstra J, Berrens L, Bruynzeel PL: The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch Dermatol Res* 278:199–204, 1986
3. Leung DYM, Schneeberger EE, Siraganian RP, Geha RS, Bhan AK: The presence of IgE on monocytes/macrophages infiltrating the skin lesions of atopic dermatitis. *Clin Immunol Immunopathol* 42:328–337, 1987
4. Bruynzeel-Koomen C, van der Donk EMM, Bruynzeel PLB, Capron M, de Gast GC, Mudde G: Associated expression of CD1 antigen and Fc receptor for IgE on epidermal Langerhans cells from patients with atopic dermatitis. *Clin Exp Immunol* 74:137–142, 1988
5. Bieber T, Rieger A, Neuchrist C, Prinz JC, Rieber EP, Boltz-Nitulescu G, Scheiner O, Kraft D, Ring J, Stingl G: Induction of FcεRII/CD23 on human epidermal Langerhans cells by human recombinant interleukin 4 and γ interferon. *J Exp Med* 170:309–314, 1989
6. Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Födinger D, Kinet JP, Stingl G: Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J Exp Med* 175:1353–1365, 1992
7. Bieber T, de la Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, Hanau D, de la Salle C: Human epidermal Langerhans cells express the high-affinity receptor for immunoglobulin. E (FcεRI). *J Exp Med* 175:1285–1290, 1992
8. Haas N, Hamann K, Grabbe J, Cremer B, Czarnetzki BM: Expression of the high affinity IgE-receptor on human Langerhans cells. *Acta Derm Venereol (Stockh)* 72:271–272, 1992
9. Osterhoff B, Rappersberger C, Wang B, Koszik F, Ochiai K, Kinet JP, Stingl G: Immunomorphological characterization of FcεRI-bearing cells within the human dermis. *J Invest Dermatol* 102:315–320, 1994
10. Ravetch JV, Kinet J-P: Fc receptors. *Annu Rev Immunol* 9:547–592, 1991
11. Miettinen HM, Rose JK, Mellman I: Fc receptor isoforms exhibit distinct abilities for coated pit localization as a result of cytoplasmic domain heterogeneity. *Cell* 58:317–326, 1989
12. Amigorena S, Salamero J, Davoust J, Fridman WH, Bonnerot C: Tyrosine-containing motif that transduces cell activation signals also determines internalization and antigen presentation via type III receptors for IgG. *Nature* 358:337–341, 1992
13. Caux C, Dezutter-Dambuyant C, Schmitt D, Bancherau J: GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. *Nature* 360:258–261, 1992
14. Sallusto F, Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte-macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 179:1109–1118, 1994
15. Romani N, Gruner S, Brang D, Kämpgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM, Schuler G: Proliferating dendritic cell progenitors in peripheral blood. *J Exp Med* 180:83–93, 1994
16. Dessaint JP, Capron A: Fc_ϵ receptor II-positive macrophages and platelets: potent effector cells in allergy and defense against helminth parasites. *Semin Immunopathol* 12:349–363, 1990
17. Liu FT, Frigeri LT, Gritzmacher CA, Hsu DK, Robertson MW, Zuberi RI: Expression and function of an IgE-binding animal lectin (εBP) in mast cells. *Immunopharmacology* 26:187–195, 1993
18. Maurer D, Fiebiger E, Reininger B, Wolff-Winiski B, Jouvin M-H, Kilgus O, Kinet J-P, Stingl G: Expression of functional high affinity IgE receptors (FcεRI) on monocytes of atopic individuals. *J Exp Med* 179:745–750, 1994
19. Alber G, Miller L, Jelsema C, Varin-Blank N, Metzger H: Structure/function relationships of the mast cell high-affinity receptor for IgE (FcεRI). *J Biol Chem* 266:22613–22620, 1991
20. Mao SY, Alber G, Rivera J, Kochan J, Metzger H: Interaction of aggregated native and mutant IgE receptors with the cytoskeleton. *Proc Natl Acad Sci USA* 89:222–226, 1992
21. Reth M: Antigen receptor tail clue. *Nature* 338:383–384, 1989
22. Jouvin MHE, Adamczewski M, Numerof R, Letourneur O, Valle A, Kinet JP: Differential control of the tyrosine kinases lyn and syk by the two signaling chains of the high affinity immunoglobulin E receptor. *J Biol Chem* 269:5918–5925, 1994
23. Bieber T: FcεRI on human Langerhans cells: a receptor in search of new functions. *Immunol Today* 15:52–53, 1994
24. Ishizaka K: Basic mechanism of IgE-mediated hypersensitivity. *Curr Opin Immunol* 4:625–629, 1989
25. Orning L, Hammarstrom S, Samuelsson B: Leukotriene D: a slow reacting substance from rat basophilic leukemia cells. *Proc Natl Acad Sci USA* 77:2014–2017, 1980
26. Ortega C, Hazan B, Zor U, Pecht I: Mast cell stimulation by monoclonal antibodies specific for the Fc_ϵ receptor yields distinct responses of arachidonic acid and leukotriene C_4 secretion. *Eur J Immunol* 19:2251–2257, 1989
27. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE: Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophor. *Nature* 339:64–67, 1989
28. Wodnar-Filipovicz A, Heusser CH, Moroni C: Production of the hemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. *Nature* 339:150–152, 1989
29. Burd RP, Rogers HW, Gordon JR, Martin CA, Jayaraman S, Wilson SD, Dvorak AM, Galli SJ, Dorf ME: Interleukin-3 dependent and independent mast cells stimulated with IgE and antigen express multiple cytokines. *J Exp Med* 170:245–257, 1989
30. Heufler C, Topar G, Koch F, Trockenbacher B, Kämpgen E, Romani N, Schuler G: Cytokine gene expression in murine epidermal cell suspensions: interleukin 1β and macrophage inflammatory protein 1α are selectively expressed in

Langerhans cells but are differently regulated in culture. *J Exp Med* 176:1221–1226, 1992

31. Matsue H, Cruz PD Jr, Bergstresser PR: Langerhans cells are the major source of mRNA for IL-1β and MIP-1α among unstimulated mouse epidermal cells. *J Invest Dermatol* 99:537–541, 1992
32. Schreiber S, Kilgus O, Payer E, Kutil R, Elbe A, Müller C, Stingl G: Cytokine pattern of Langerhans cells isolated from murine epidermal cell cultures. *J Immunol* 149:3524–3534, 1992
33. Nathan CF: Secretory products of macrophages. *J Clin Invest* 79:319–326, 1987
34. Borish L, Mascali JJ, Rosenwasser LJ: IgE-dependent cytokine production by human peripheral blood mononuclear phagocytes. *J Immunol* 146:63–67, 1991
35. Ferreri NR, Howland WC, Spiegelberg HL: Release of leukotrienes C4 and B4 and prostaglandin E2 from human monocytes stimulated with aggregated IgG, IgA, and IgE. *J Immunol* 136:4188–4193, 1986
36. Rankin JA, Hitchcock M, Marrill WW, Bach MK, Brashler JR, Askenase PW: IgE-dependent release of leukotriene C_4 from alveolar macrophages. *Nature* 297:329–331, 1982
37. Leung DYM, Pober JS, Cotran RS: Expression of endothelial leukocyte adhesion molecule-1 (ELAM-1) in elicited late phase allergic reactions. *J Clin Invest* 87:1805–1809, 1991
38. Mullarkey MF, Leiferman KM, Peters MS, Caro I, Roux ER, Hanna RK, Rubin AS, Jacobs CA: Human cutaneous allergic late-phase response is inhibited by soluble IL-1 receptor. *J Immunol* 152:2033–2041, 1994
39. Petzelbauer P, Bender J, Wilson J, Pober JS: Heterogeneity of dermal microvascular endothelial cell antigen expression and cytokine responsiveness in situ and in cell culture. *J Immunol* 151:5062–5072, 1993
40. Springer TA: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76:301–314, 1994
41. Van der Heijden FC, Wierenga EA, Bos JD, Kapsenberg JL: High frequency of IL-4 producing $CD4^+$ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 97:389–394, 1991
42. Kay AB, Ying S, Varney V, Gaga M, Durham SR, Moqbel R, Wardlaw AJ, Hamid Q: Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 173:775–778, 1991
43. Hamid Q, Boguniewicz M, Leung DYM: Differential in situ gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 94:870–876, 1994
44. Müller KM, Jaunin F, Masouyé I, Saurat J-H, Hauser C: Th2 cells mediate IL-4-dependent local tissue inflammation. *J Immunol* 150:5576–5584, 1993